Supplementary Materials:

Materials and Methods

Effects of F-actin filament polymerization on ABCB1a translocation at fertilization.
The dynamics of actin polymerization and depolymerization on ABCB1a protein localization in embryos was examined by whole mount immunolocalization. Eggs were obtained, washed, fertilized and the fertilization envelopes were removed as described in the section entitled *Sea urchin collection and embryo culture*. Embryos were cultured in CaFSW at 13-15^{o}C until fixation. At 10 and 50 min PF embryos were exposed to 2 µM of cytochalasin D, latrunculin A, jasplakinolide or DMSO (solvent control). Actin inhibitors were dissolved in DMSO and the inhibitor volume never exceeded 0.2% of the embryo culture volume. At 60 min PF embryos were fixed in 3.2% paraformaldehyde and 0.3% glutaraldehyde in MPBS (pH 7.0) for 30 min at room temperature. Samples were processed through a series of primary (Ab1-C1 at 1:30) and secondary antibody (1:500) incubations as described in the section entitled *Immunolocalization of ABCB1a* and imaged by confocal.

Effects of F-actin bundle formation on activation of transport and ABCB1a localization.
Eggs were diluted to 1000 eggs/ml for use in all treatments. For treatments that involved fertilization, the eggs were dejellied in acidified FSW (pH 4.8) for 3 min and were fertilized in a final dilution of 1:375,000 dry sperm. The intracellular changes in calcein accumulation in embryos were measured using a Zeiss LSM-700 laser scanning confocal microscope (Jena, Germany) equipped with a Zeiss Plan APOChromat 20x 0.8NA air objective equipped with a cooling stage insert to keep embryos chilled at 14-15^{o}C. Approximately 1000 embryos in 1 ml were placed in the bottom of a Delta-T tissue culture dish and covered by a glass lid to prevent evaporation (Bioptechs, Butler, PA, USA). Fluorescence intensity measurements were obtained using ImageJ software.

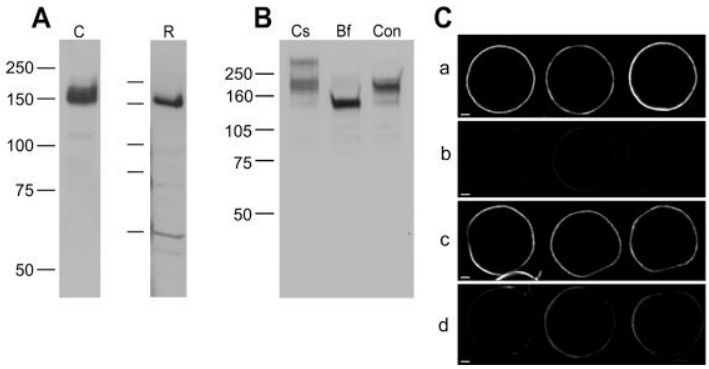
Egg activation with sperm and the resulting exocytosis of the cortical granules, membrane elevation, membrane conductance changes, respiratory burst and increases in protein and DNA synthesis, can be mimicked *in vitro* with micromolar amounts of the divalent ionophore A23187 (Steinhardt and Epel, 1974). To examine the impact of ionophore activation alone on ABC transporter activity, including the contribution of sperm to ABC transporter-mediated calcein efflux, eggs were parthenogenetically activated in 30 µM of calcium ionophore A23187, incubated at 13-15^{o}C, and exposed to 250 nM calcein-AM at both 60 min and 120 min post-activation for 1 h, and were then imaged on the confocal as described above.

Previous work has demonstrated the formation of actin bundles in elongated microvilli and actin rootlet formation after fertilization requires the Na+-dependent alkalinization of the cytoplasm. Cytoplasmic alkalinization is prevented in parthenogenetically activated eggs with A23187 in NaFFSW resulting in delayed microvilli development and the lack

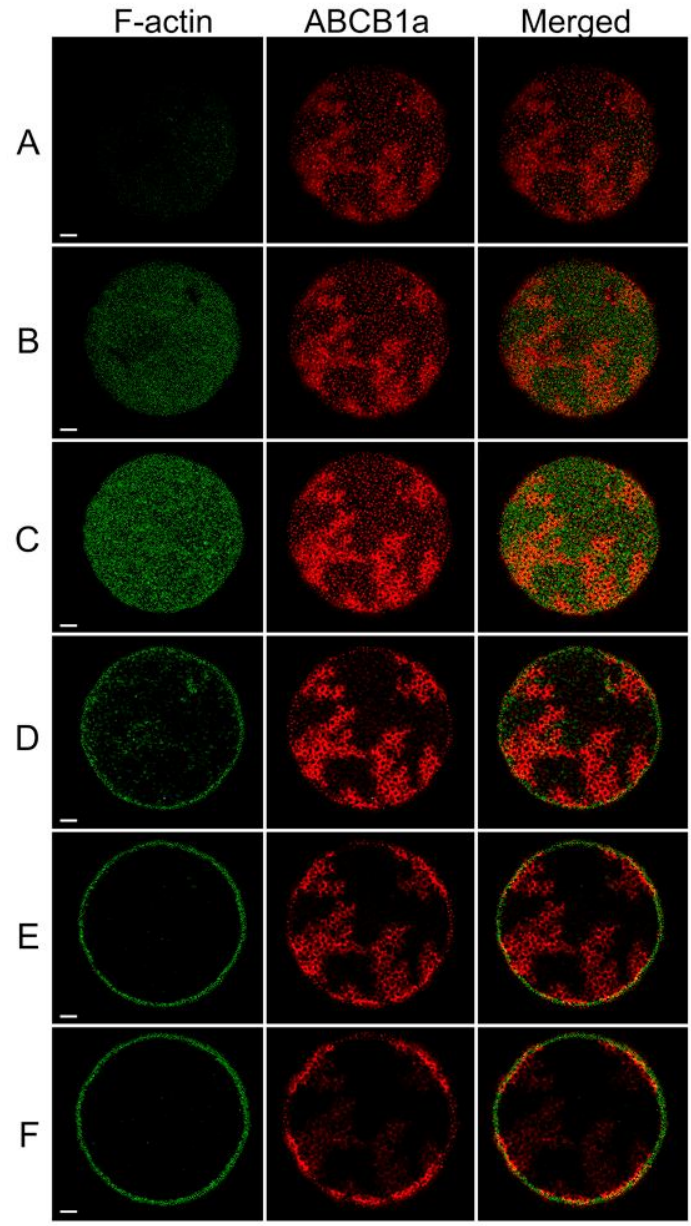
of actin rootlet formation. To investigate whether actin microvillar bundle and rootlet formation are required for ABC transporter activity, eggs were dejellied in acidified NaFFSW (pH 4.8), then washed twice in NaFFSW (pH 8.0), parthenogenetically activated in 30 µM calcium ionophore A23187 and incubated at 13-15^{o}C. Embryos were then exposed to 250 nM calcein-AM for 1 h at both 60 min and 120 min post-fertilization/activation and imaged as described above. Upon addition of 40 mM NaCl to A23187 activated eggs cultured in NaFFSW, cytoplasmic alkalinization will occur and actin rootlet formation will develop over a time course similar to that of control eggs . To determine the effect of cytoplasmic alkalinization in A23187 activated embryos on ABC transporter activity an additional batch of ionophore-activated embryos were exposed to NaCl at a final concentration of 40 mM at 60 min post-activation, then exposed to 250 nM calcein-AM for 1 h at 120 min post-activation, and imaged by confocal. Ten embryos from ten females (batches) were measured for each treatment and control condition.

To investigate the localization of ABCB1a in ionophore activated embryos grown in NaFFSW, eggs were activated and cultured as described above and fixed at 60 min PF in 3.2% paraformaldehyde and 0.3% glutaraldehyde in MPBS (pH 7.0) for 30 min at room temperature. Samples were processed through a series of primary (Ab1-C1 at 1:30) and secondary antibody (1:500) incubations as detailed in the section entitled *Immunolocalization of ABCB1a* described above and imaged by confocal.

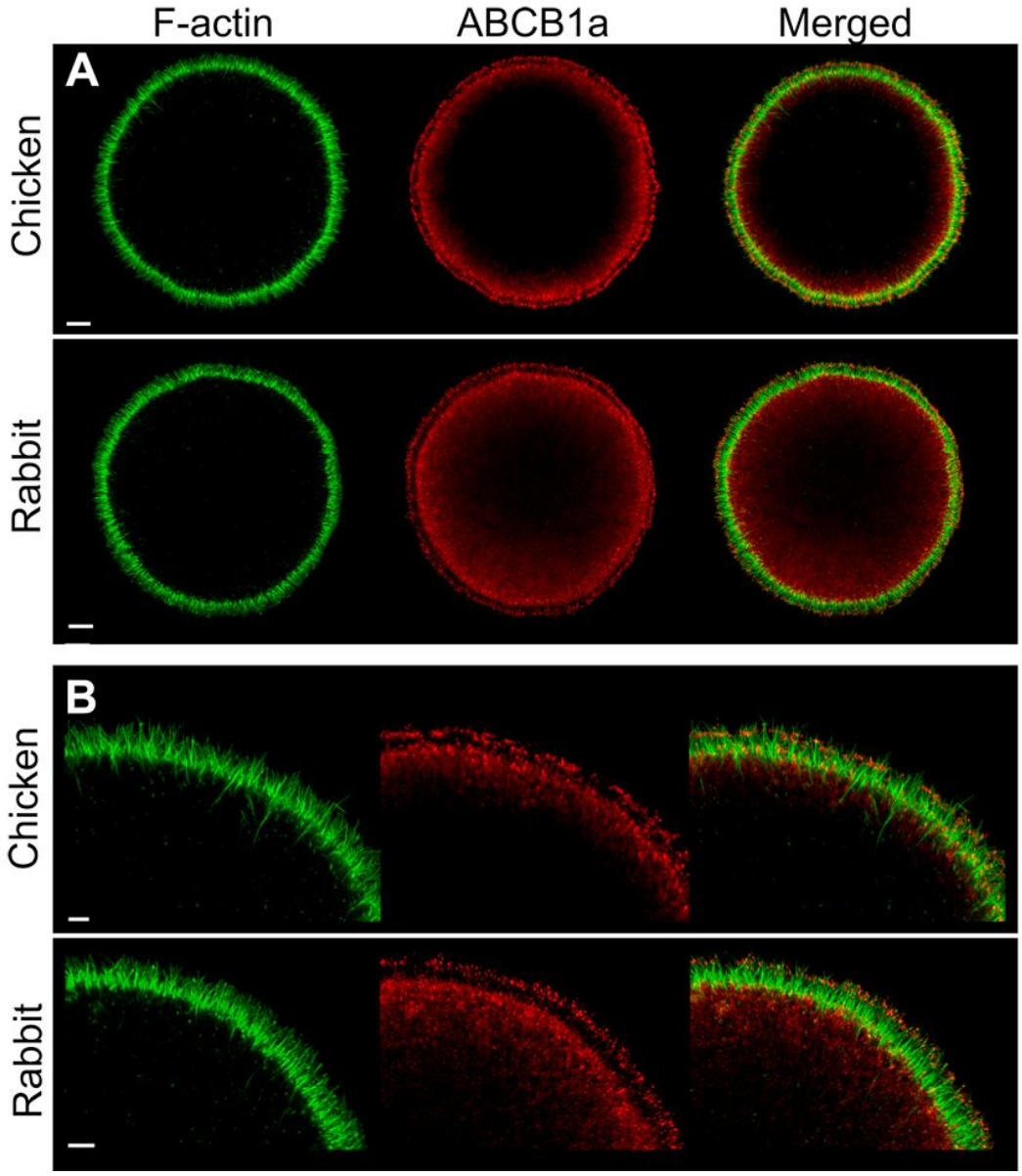
Steinhardt, R. A. and Epel, D. (1974) Activation of sea-urchin eggs by a calcium ionophore. Proc. Natl. Acad. Sci. 71, 1915-1919.



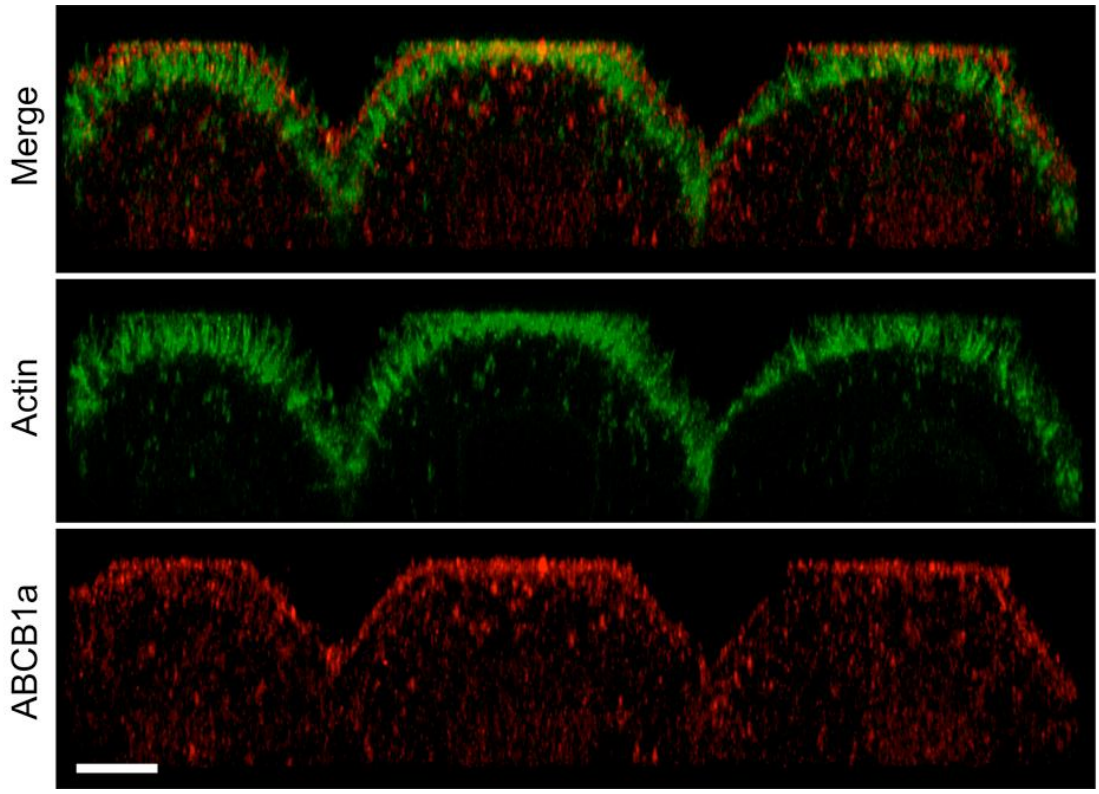
Supplemental Figure 1. Characterization of sea urchin ABCB1a glycoforms and antibodies. (A) Isolated insect cell microsomes expressing *Strongylocentrotus purpuratus* ABCB1a were probed with both polyclonal anti-peptide antibodies to *S. purpuratus* ABCB1a (Ab1-C1 and Ab4-R2) produced in chicken (C) and rabbit (R). (B) Glycoforms of ABCB1a were determined by microinjecting embryos with mRNA encoding *S. purpuratus* ABCB1a and incubating these embryos in 5 µM brefeldin-A (Bf), representing the non-glycosylation state of the protein, or 10 µM cyclosporin-A (Cs), inducing hyperglycosylation, and loading 80-100, 18 h old embryos per lane (a level at which endogenous ABCB1a is not detectable) and probing with Ab1-C1. Control embryos (Con). (C) Unfertilized eggs were incubated with Ab1-C1 (0.03 mg/mL, panel A), or non-immune chicken IgY (0.03 mg/mL, panel B). For peptide competition experiments, Ab1-C1 was pre-incubated with 100x molar excess of either a random peptide corresponding to an alternative region of ABCB1a (panel C), or the synthetic peptide recognized by Ab1-C1 (panel D) resulting in the loss of all immunodetection.



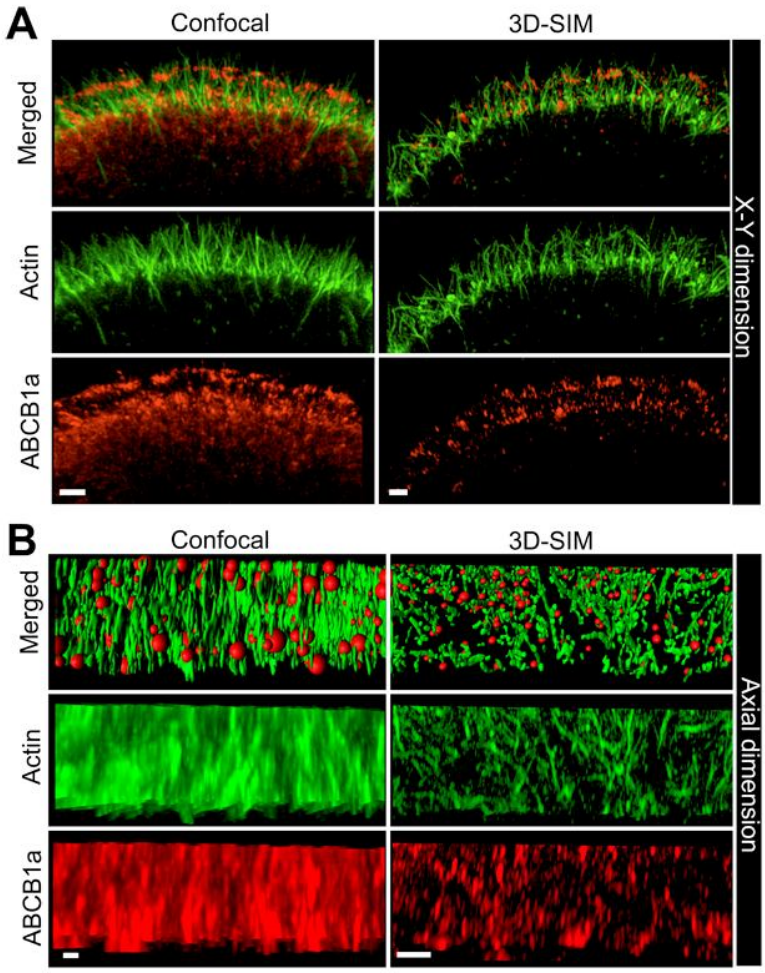
Supplemental Figure 2. Large pools of ABCB1a protein are present just below the microvilli in the unfertilized egg. Serial tangential sectioning of the egg surface through the cortex (A to F in direction). Cells were stained for actin (green) with phalloidin and ABCB1a (red) with Ab1-C1 (0.03 mg/mL). Microvillar staining is primarily located in panels B and C, while the majority of ABCB1a protein can be localized to the base of the microvilli or below in large pools (panels C, D and E). Negative staining in the ABCB1a pools corresponds to cortical granules. Scale bar = 4 μm.



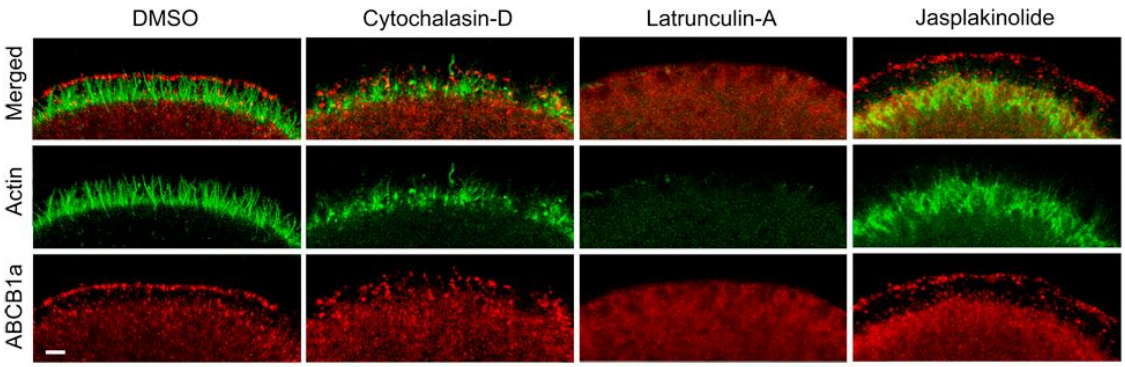
Supplemental Figure 3. Microvillar tip localization of ABCB1a is confirmed by a second antibody. Polyclonal anti-peptide antibodies to *S. purpuratus* ABCB1a produced in chicken, Ab1-C1 (0.03 mg/mL) and rabbit, Ab4-R2 (0.005 mg/mL) were used to stain embryos. Actin was stained with (AlexaFluor 488)-phalloidin. (A) Cross section through an embryo at 60 min PF. Scale bar = 8µm. (B) Detailed cross section of the embryo cortex and microvillar surface at 60 min PF. Scale bar = 3µm



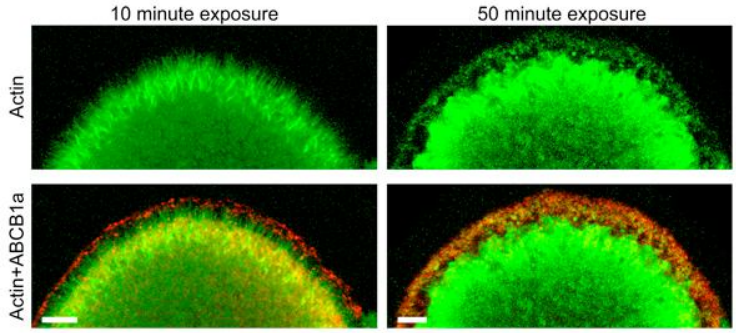
Supplemental Figure 4. Localization of ABCB1a in mesomeres of 32-cell embryos. Embryos were stained for actin (green, phalloidin) and ABCB1a (red, Ab1-C1, 0.03 mg/mL). Cross section of the embryo surface visualized by confocal microscopy. Scale bar = 5 µm.



Supplemental Figure 5. Improved axial resolution using 3D-structured illumination microscopy. Embryos (60 min PF) were stained for actin (green, phalloidin) and ABCB1a (red, Ab1-C1 at 0.03 mg/mL). (A) Cross section of the embryo surface visualized by confocal and 3D-SIM in the X-Y dimension. Scale bar = 2 µm. (B) Axial view (Z-direction) looking down on the microvillar surface of an embryo. Isosurfaces of the actin filament and ABCB1a spots were generated with Imaris software and are shown in the panel labeled merge. Scale bar = 2 µm.



Supplemental Figure 6. ABCB1a translocation is affected by actin inhibitors. Embryos were exposed and cultured in 2 µM jasplakinolide, cytochalasin-D, latrunculin-A and solvent control (DMSO) at 50 min PF and imaged at 60 min PF. Embryos were stained for actin (green, phalloidin) and ABCB1a (red, Ab1-C1). Jasplakinolide facilitates actin polymerization and F-actin stabilization causing the increased accumulation of ABCB1a in the cell cortex. Scale bar = 2 µm.



Supplemental Figure 7. Visualization of microvilli in jasplakinolide exposed embryos. Jasplakinolide induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin. To enhance the detection of microvilli in jasplakinolide exposed embryos stained with phalloidin from Figure 5 and Supplemental Figure 6, the fluorescence intensity was enhanced. Scale bar = 8 µm.